This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See below for additional information.

Interagency Report Control No 0180-DOA-AN

SH

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. Registration No: 57-F-0004
Customer No: 947

FORM APPROVED
OMB NO. 0579-0036

2. Headquarters Research Facility *(Name and Address, as registered with USDA, include Zip Code)*:

Centers for Disease Control and Prevention
1600 Clifton Road, NE
(b)(2)High, (b)(7)f
Atlanta, GA 30333

RECEIVED
NOV 19 2009

3. Reporting Facility (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS *(sites)* **- See Attached Listing (A)**

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report.)*	F. TOTAL No. OF ANIMALS (Cols. C + D + E)
4. Dogs	0	433	6	0	439
5. Cats	0	6	0	0	6
6. Guinea Pigs	0	32	186	0	218
7. Hamsters	0	1205	446	49	1700
8. Rabbits	0	108	56	0	164
9. Non-Human Primates	0	439	204	8	651
10. Sheep	0	170	0	0	170
11. Pigs	0	312	0	0	312
12. Other Farm Animals	See APHIS Form 7023A				
13. Other Animals					

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report**. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

Signature of C.E.O. or Institutional Official	Name & Title of C.E.O. or Institutional Official	Date Signed:
(b)(6), (b)(7)c		11/17/09

EG 12/3/09

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Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. Registration No: 57-F-0004
Customer No: 947

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OMB NO. 0579-0036

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1600 Clifton Road NE
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Atlanta, GA 30333

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report.)*	F. TOTAL No. OF ANIMALS (Cols. C + D + E)
Cattle	0	521	0	0	521
Goat	0	425	0	0	425
Dormouse	0	2	6	0	8
Squirrel	0	0	110	0	110
House Rat	0	0	583	0	583
Grasshopper mouse	0	0	158	0	158
Oppossum	0	0	1	0	1
White-footed mouse	0	0	82	0	82
Deer Mouse	0	614	603	0	1217
Bat	0	102	6	1	109
Ferret	0	893	0	31	924
Fox	0	7	24	1	32
Gambian Rat	0	0	9	0	9
Gerbil	0	2	50	0	52

ASSURANCE STATEMENTS

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2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report**. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

Signature of C.E.O. or Institutional Official	Name & Title of C.E.O. or Institutional Official	Date Signed:
(b)(6), (b)(7)c		11/17/09

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Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. Registration No: 57-F-0004
Customer No: 947

FORM APPROVED
OMB NO. 0579-0036

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Centers for Disease Control and Prevention
1600 Clifton Road NE
(b)(2)High, (b)(7)f
Atlanta, GA 30333

NOV 19 2009

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report.)*	F. TOTAL No. OF ANIMALS (Cols. C + D + E)
Meadow Vole	0	31	53	0	84
Mountain Vole	0	5	0	0	5
Cinereus shrew	0	2	20	0	22
Chipmunk	0	2	111	0	113
Western jumping mouse	0	1	0	0	1
Woodrat	0	2	36	0	38
Prairie Dog	0	385	52	25	462
Raccoon	0	7	7	0	14
Striped skunk	0	0	1	0	1
Heart-nosed bat	0	5	0	0	5
Pipistrelle bat	0	79	0	0	79
African sheath bat	0	60	0	0	60
Straw coloured fruit bat	0	4	0	0	4

ASSURANCE STATEMENTS

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2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report**. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

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(b)(6), (b)(7)c		11/17/09

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Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

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(TYPE OR PRINT)

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Customer No: 947

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OMB NO. 0579-0036

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1600 Clifton Road NE
(b)(2)High, (b)(7)f
Atlanta, GA 30333

NOV 19 2009

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Ethiopian epauletted fruit bat	0	18	0	0	18
Wahlberg's epauletted fruit bat	0	35	0	0	35
Giant leaf-nosed bat	0	9	0	0	9
Leaf-nosed bat	0	29	0	0	29
Angolan fruit bat	0	10	0	0	10
Greater long-fingered bat	0	2	0	0	2
Least long-fingered bat	0	66	0	0	66
Bent wing bat	0	108	0	0	108
White-winged serotine	0	33	0	0	33
Slit-faced bat	0	18	0	0	18

ASSURANCE STATEMENTS

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CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

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(b)(6), (b)(7)c		11/17/09

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Interagency Report Control No 0180-DOA-AN

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1600 Clifton Road NE
(b)(2)High, (b)(7)f
Atlanta, GA 30333

NOV 19 2009

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Hidelbrandt's horsheshoe bat	0	60	0	0	60
Lander's horseshoe bat	0	16	0	0	16
African yellow bat	0	9	0	0	9
Hildegarde's tomb bat	0	1	0	0	1
Persian trident bat	0	15	0	0	15
Lesser brown horseshoe bat	0	88	0	0	88
Great roundleaf bat	0	33	0	0	33
Intermediate roundleaf bat	0	38	0	0	38
Black-bearded tomb bat	0	4	0	0	4
Wrinkled-lipped bat	0	70	0	0	70

ASSURANCE STATEMENTS

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CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

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(b)(6), (b)(7)c		11/17/09

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Stoliczka's trident bat	0	10	0	0	10
Short-tailed fruit bat	0	2	0	0	2
Yellow-shouldered bat	0	1	0	0	1
Caribbean white bat	0	24	0	0	24
Mexican fruit bat	0	15	0	0	15
Great fuit eating bat	0	3	0	0	3
Toltec fruit eating bat	0	1	0	0	1
Chestnut short-tailed bat	0	1	0	0	1
Seba's short tailed bat	0	19	0	0	19
Nectar feeding bat	0	19	0	0	19

ASSURANCE STATEMENTS

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4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

Signature of C.E.O. or Institutional Official	Name & Title of C.E.O. or Institutional Official	Date Signed:
(b)(6), (b)(7)c		11/17/09

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Big-eared bat	0	25	0	0	25
Elegant bat	0	3	0	0	3
Pale spear-nosed bat	0	9	0	0	9
Hellar's broad-nosed bat	0	3	0	0	3
Davy's naked-backed bat	0	19	0	0	19
Long-tongued dawn fruit bats	0	49	0	0	49
Lesser short-nosed fruit bat	0	41	0	0	41
Lesser dawn bat	0	1	0	0	1
Greater musky fruit bat	0	22	0	0	22
Egyptian fruit bat	0	813	0	0	813

ASSURANCE STATEMENTS

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CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

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Geoffrey's rousette	0	1	0	0	1
Lesser Asiatic yellow bat	0	6	0	0	6

ASSURANCE STATEMENTS

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Signature of C.E.O. or Institutional Official	Name & Title of C.E.O. or Institutional Official	Date Signed:
(b)(6), (b)(7)c		11/17/09

Protocol: 1593FRAHAMC
Species (common name): Hamster
Number: 49
Explanation of procedure producing pain and/or distress:
The development of clinical rabies infection is expected to occur in a subset of the infected animals. Based on prior experience with hamsters under similar experimental protocols we might expect up to 10% of animals that develop signs of rabies that progress to a terminal state before euthanasia can be administered.
Justification why pain and/or distress could not be relieved:
All animals infected with rabies virus will be euthanized at first onset of clinical signs of rabies. Beginning 7 days post infection animals will be examined at least twice daily by rabies staff and *ad hoc* by animal care staff during routine husbandry so that euthanasia can be promptly administered. However, occasionally animals progress rapidly from apparently healthy to a terminal state. This can occasionally occur overnight or between routine check periods. The majority of animals will be euthanized at first onset and we do not expect a significant number of animals to rapidly progress as described above.

Protocol: 1608DONFERC
Species (common name): Ferret
Number: 11
Explanation of procedure producing pain and/or distress:
Infection of ferrets with highly pathogenic H5N1 or H7N7 virus may cause severe morbidity, including neurological symptoms and death. Therefore, we believe that a Category E pain class is warranted in cases where animals exhibit severe respiratory symptoms as well as multiple organ dysfunction.
Justification why pain and/or distress could not be relieved:
Two kinds of drugs were considered: 1) Non-steroidal anti-inflammatory drugs (NSAIDs, COX inhibitors) and related analgesics. This class of drugs can not be used because they may alter viral replication levels, body temperature and body weight loss; all of which are important parameters in our studies. NSAID therapy is not considered because of its potentially detrimental impact on the interpretation of the effects of the vaccine. 2) Opioid drugs. These agents cause depression of respiratory control centers in the CNS. Highly pathogenic influenza virus infection in ferrets can cause an acute respiratory distress syndrome which may be aggravated by opioid drugs. Consequently, opioid therapy is not considered because of its potentially detrimental impact on the interpretation of the effects of the vaccine. Pain will be minimized by observation of the animals as frequently as 4-hour intervals to evaluate the severity of the symptoms and make the earliest possible decision (according to predetermined criteria) on humane termination by euthanasia.

Protocol: 1643RUPBATL
Species (common name): Bat
Number: 1
Explanation of procedure producing pain and/or distress:
The development of clinical rabies infection is expected to occur in a subset of the infected animals. There are occasionally animals that progress to a terminal state before we are able to humanely euthanize them. However, it is critical to understand infection dynamics in this important rabies reservoir.
Justification why pain and/or distress could not be relieved:

All animals infected with rabies virus will be euthanized at first onset of clinical signs of rabies. Beginning 7 days post infection animals will be examined at least daily by rabies staff and *ad hoc* by animal care staff during routine husbandry so that euthanasia can be promptly administered. However, occasionally animals progress rapidly from apparently healthy to a terminal state. This can occasionally occur overnight or between routine check periods. The majority of animals will be euthanized at first onset and we do not expect a significant number of animals to rapidly progress as described above.

Protocol: 1652RUPFOXL
Species (common name): Fox
Number: 1
Explanation of procedure producing pain and/or distress:
The development of clinical rabies infection is expected to occur in a subset of the infected animals. There are occasionally animals that progress to a terminal state before we are able to humanely euthanize them. However, it is critical to understand infection dynamics in this important rabies reservoir.
Justification why pain and/or distress could not be relieved:
All animals infected with rabies virus will be euthanized at first onset of clinical signs of rabies. Beginning 7 days post infection animals will be examined at least daily by rabies staff and *ad hoc* by animal care staff during routine husbandry so that euthanasia can be promptly administered. However, occasionally animals progress rapidly from apparently healthy to a terminal state. This can occasionally occur overnight or between routine check periods. The majority of animals will be euthanized at first onset and we do not expect a significant number of animals to rapidly progress as described above.

Protocol: 1683DAMPRAC
Species (common name): Prairie Dog
Number: 25
Explanation of procedure producing pain and/or distress:
Animals will be anesthetized during sampling to avoid pain and discomfort. However, pain associated with infection will not be treated with anesthetics or analgesics such as metacam because metacam is shown to interfere with the Nuclear Factor Kappa B (inflammatory pathway) which is believed to be an important process for pox virus infections.
Justification why pain and/or distress could not be relieved:
The nuclear factor ?B (NF-?B) transcription factor is involved in the transcription of many proinflammatory as well as antiapoptotic genes and therefore is an important component in the progression of inflammatory diseases, including poxviruses such as monkeypox (Barnes et al 1997). Orthopoxviruses appear to have acquired mechanisms to inhibit antiviral effects of NF-?B activation; encoding multiple proteins that act in various ways to prevent NF-?B activation (Reviewed by Moss and Shisler 2001; Seet et al 2003). The ability of orthopoxviruses to modulate NF-?B activation likely plays an important role in the ability of these viruses to cause disease, and therefore the utilization of substances that inhibit NF-?B activation would greatly change the normal virus lifecycle. Numerous drugs have been shown to inhibit NF-?B and therefore have anti-inflammatory results in experimental models (Reviewed by D'Acquisto, May and Ghosh 2002). These include salicylates, NSAIDs, and glucocorticoids to name a few. Metacam is commonly prescribed by veterinarians and falls into the NSAID category. Because this study is aimed at better understanding the pathogenesis of the two monkeypox strains, including through the use of recombinant viruses, it is important that the life cycle of the two

virus strains as well as wild-type and recombinant viruses not be altered by the use of NSAIDs or other anti-inflammatory agents. References:

1) (b)(6), (b)(7)c 1997. Nuclear factor- ?B: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 336: 1066-1071.

2) (b)(6), (b)(7)c 2001. Immunology 101 at poxvirus U: immune evasion genes. Semin Innumol 13(1): 59-66.

3) Seet, B.T., et al. Poxviruses and immune evasion. 2003. Annu Rev Immunol 21: 377-423.

4) (b)(6), (b)(7)c 2002. Inhibition of nuclear factor kappa B (NF-?B): an emerging theme in anti-inflammatory therapies. Molec Interven 2: 22-35.

Protocol: 1701MAIFERC
Species (common name): Ferret
Number: 5
Explanation of procedure producing pain and/or distress:
Ferrets are used to model influenza virus infection because they respond in similar ways as humans when infected and they have similar distribution of receptors in their respiratory tracts as humans. Infection of ferrets with highly pathogenic H5N1 viruses may cause severe morbidity and even death in some cases. Every attempt will be made to euthanize the animal prior to it reaching a moribund state. Animals will be monitored daily and will be euthanatized if they reach 10 points on the 10-point euthanasia scale.
Justification why pain and/or distress could not be relieved:
The crux of our study is to understand the mechanisms of disease caused by influenza virus infection. Disease is assessed based on clinical signs observed in infected animals such as lethargy, fever, weight loss, etc… that would be obscured by pain relieving drugs. After influenza virus infection, the initial inflammatory response, as well as the subsequent adaptive T and B cell responses, are crucial to the progression of disease and directly affects the clinical signs observed in the animals (1,2). Any manipulation of the inflammatory response with analgesics will affect the overall immune response and will prevent an accurate assessment of disease. For example, Type I and II interferon and NK cell responses are important components of the immune response to influenza. Manipulation of these responses with anti-inflammatory drugs such as NSAIDS or opioids directly affect virus shedding, incidence of fever and additional disease parameters (3-6). Our preliminary studies suggest that a majority of viruses to be evaluated in this study will actually be attenuated for ferrets. Therefore, we estimate that only 25% of animals at most may experience symptoms that require euthanasia.

1. Immunol Rev. Pathogenesis of emerging avian influenza viruses in mammals and the host innate immune response. 2008 Oct;225:68-84.
2. Emerg Infect Dis. Antiviral response in pandemic influenza viruses. 2006 Jan;12(1):44-7.
3. J Virol. Intranasal administration of alpha interferon reduces seasonal influenza A virus morbidity in ferrets. 2009 Apr;83(8):3843-51.
4. Curr Med Res Opin. Alleviating flu-like symptoms with dose titration and analgesics in MS patients on intramuscular interferon beta-1a therapy: a pilot study.2007 Jul;23(7):1667-72.
5. Life Sci. Morphine reduces pulmonary inflammation in response to influenza infection. 1999;65(11):1141-52.
6. Brain Behav Immun. Stress-induced modulation of NK activity during influenza viral infection: role of glucocorticoids and opioids. 2005 Mar;19(2):153-64.

Protocol: 1717TUMFERC
Species (common name): Ferret
Number: 7
Explanation of procedure producing pain and/or distress:
Ferrets are used to model influenza virus infection because they are susceptible to virus infection and have a course of disease that is similar to humans. It is generally believed that some pain and/or distress will occur when ferrets are infected with influenza strains that are considered highly pathogenic. During FY 2009, under this protocol, some ferrets were infected with highly pathogenic influenza strains which cause severe morbidity and even death in some cases. Every attempt was made to euthanize the animal prior to the development of severe disease. Animals were monitored twice daily and euthanized when they reached 10 points on a 10-point euthanasia scale.
Justification why pain and/or distress could not be relieved:
It has been demonstrated that analgesic drugs or anti-inflammatory agents affect the respiratory inflammation during influenza infections (references on form). We have chosen not to use pain relieving drugs because they can have serious consequences towards the outcome of virus infection. The main purpose of this animal protocol is to better understand the mechanisms of disease cause by highly pathogenic influenza virus infection. Disease is assessed based on clinical signs observed among infected animals such as lethargy, fever, weight loss, leucopenia and cytokine production, many of which would be obscured by pain relieving drugs. After influenza virus infection, the initial acute inflammatory response, as well as subsequent adaptive T and B cell responses are crucial to the progression of disease and directly affects the clinical signs observed in the animals. Any manipulation of the inflammatory responses with analgesics will affect the acute assessment of the disease. Moreover, manipulation of these responses with anti-inflammatory drugs such as NSAIDS or opioids directly affect virus shedding, incidence of fever, and additional disease parameters as indicated in references. It is also important to note that this animal model is being used to model human influenza virus infection where in most cases; infected individuals will not be taking NSAIDS or opioids. Thus, the data obtained from treated animals would not model the real-life situation. Our ultimate goal is to understand the unmanipulated disease process so we can more precisely target strategies of treatment.
1. Immunol Rev. Pathogenesis of emerging avian influenza viruses in mammals and the host innate immune response. 2008 Oct;225:68-84.
2. Emerg Infect Dis. Antiviral response in pandemic influenza viruses. 2006 Jan; 12(1):44-7.
3. J Virol. Intranasal administration of alpha interferon reduces seasonal influenza A virus morbidity in ferrets. 2009 Apr;83(8):3843-51.
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5. Life Sci. Morphine reduces pulmonary inflammation in response to influenza infection. 1999; 65(11):1141-52.
6. Brain Behav Immun. Stress-induced modulation of NK activity during influenza viral infection: role of glucocorticoids and opioids. 2005 Mar; 19(2):153-64.

Protocol: 1720KLIFERC
Species (common name): Ferret
Number: 4
Explanation of procedure producing pain and/or distress:
The potential pain and discomfort will come from the actual progression of the highly pathogenic avian influenza virus in only a few animals.
Justification why pain and/or distress could not be relieved:

Viruses that exhibit virulence in ferrets may cause substantial respiratory disease and death on days 7-10 post-infection. Some animals retained through the second week of infection may develop neurological symptoms. Onset of neurological symptoms may occur as early as day 7 or as late as 13 days post-infection. Neurological signs include torticollis, ataxia and hind-limb paresis. When these neurological signs are observed, the animal is euthanized. Since animals are observed twice a day, ferrets would exhibit signs for less than 1 day before euthanasia. In rare cases, animals may die without exhibiting respiratory or neurological symptoms and with disease apparently no more severe than those that survive. The experiments are terminated at day 14 post-infection when all remaining animals are euthanized.

Protocol: 1732MAIFERC
Species (common name): Ferret
Number: 4
Explanation of procedure producing pain and/or distress:
Ferrets are used to model influenza virus infection because they respond in similar ways as humans when infected and they have similar distribution of receptors in their respiratory tracts as humans. Infection of ferrets with highly pathogenic H5N1 viruses may cause severe morbidity and even death in some cases. Every attempt will be made to euthanize the animal prior to it reaching a moribund state. Animals will be monitored daily and will be euthanatized if they reach 10 points on the 10-point euthanasia scale.
Justification why pain and/or distress could not be relieved:
The crux of our study is to understand the mechanisms of disease caused by influenza virus infection. Disease is assessed based on clinical signs observed in infected animals such as lethargy, fever, weight loss, etc… that would be obscured by pain relieving drugs. After influenza virus infection, the initial inflammatory response, as well as the subsequent adaptive T and B cell responses, are crucial to the progression of disease and directly affects the clinical signs observed in the animals (1, 2). Any manipulation of the inflammatory response with analgesics will affect the overall immune response and will prevent an accurate assessment of disease. For example, Type I and II interferon and NK cell responses are important components of the immune response to influenza. Manipulation of these responses with anti-inflammatory drugs such as NSAIDS or opioids directly affect virus shedding, incidence of fever and additional disease parameters (3-6). Our preliminary studies suggest that a majority of viruses to be evaluated in this study will actually be attenuated for ferrets. Therefore, we estimate that only 25% of animals at most may experience symptoms that require euthanasia.

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5. Life Sci. Morphine reduces pulmonary inflammation in response to influenza infection. 1999; 65(11):1141-52.
6. Brain Behav Immun. Stress-induced modulation of NK activity during influenza viral infection: role of glucocorticoids and opioids. 2005 Mar; 19(2):153-64.

Protocol: 1745GOFMONC
Species (common name): Cynomolgus Macaque
Number: 8
Explanation of procedure producing pain and/or distress:
Nonhuman primates were infected with Variola.

Justification why pain and/or distress could not be relieved:
Meloxicam, a long acting non-steroidal anti-inflammatory drug of the oxicam class, will be administered daily to provide analgesia, however it is expected to only provide minor relief early during disease course. Narcotics may interfere with the cytokine response of the disease in an unknown way and might invalidate the model unless additional studies were performed.

Attachment C: Exceptions to Regulations and Standards; USDA, APHIS, Form 7023 (FY09)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004
Customer Number: 947

During the reporting period, the following exceptions to the recommendations in the *Guide for the Care and Use of Laboratory Animals* were approved by the CDC – Atlanta Institutional Animal Care and Use Committee (IACUC):

1. Regarding the housing of prairie dogs assigned to research protocols, the *Guide* has recommended cage sizes for specific species of animals. However, prairie dogs are not one of the animals listed. In the *Guide*, the recommended caging requirements for our prairie dogs would fall between guinea pigs greater than 350 grams, and rabbits that are less than 2 kg. For the guinea pigs, the recommended size is greater than 101 square inches floor space and 7 inches tall. For the rabbits, 1.5 square feet of floor space and 14 inches tall. Our cages are 9 inches tall and have 216 square inches of floor space, which does fall in-between these guides. However the *Guide* also states that "at a minimum, an animal must have enough space to turn around and to express normal postural adjustments, must have ready access to food and water, and must have enough clean-bedded or unobstructed area to move and rest in". The cages we use for the prairie dogs provide all of that, with the exception of normal vertical postural adjustment. In the wild, the animals are observed to stand on their hind legs, which they are not able to do in these cages. The reason we use these cages for the prairie dogs, is because it is the largest cage manufactured that has a HEPA filtered top. This top is very important to prevent the spread of monkeypox virus between animals since this virus is believed to be spread via large respiratory droplets, fomites and/or excrement transmission. At the inception of the study design, in collaboration with staff from the CDC Animal Resources Branch, we chose these cages because our studies are typically considered short term, with animals usually remaining in these cages for 30 days or less.

2. Regarding two separate surgeries for *Saimiri*, *Aotus* and macaque monkeys, the CDC-Atlanta IACUC granted the exception for two separate surgeries in three different species of monkeys. The first surgery, a splenectomy, must occur for the animal to develop parasitemia. The second surgery is for a liver biopsy to study the liver stages. These surgeries occur in this order for the investigator to replicate the disease process.

3. Regarding sanitation of prairie dog housing, the *Guide* recommends that solid bottom enclosures are sanitized at least once weekly. The transmission study, approved by the CDC Atlanta IACUC, requires the placement of uninfected prairie dogs in a cage where an infected prairie dog was housed for 1 week. These animals remain in the dirty bedding for 3 weeks to see if they become infected from the bedding.